

11. What Is the Injurious Agent?

"To survive, a virus infects a cell and forces it to replicate; the virus uses the cell's replicative machinery to drive its own replication."

K Tanaka, et al., [164]

Single versus Multiple IAs

Atherosclerosis is a complex, chronic, inflammatory disease, characterized by a series of highly specific cellular and molecular responses, believed to be caused by multiple IAs [18,165]. Atherosclerotic lesions, however, are non-specific. There are no pathognomonic histologic features that distinguish one IA from another. If atherosclerosis is caused by multiple agents, then many different agents produce identical, highly specific, cellular and molecular responses. The development of identical or similar lesions in response to multiple different IAs can only be explained if arterial cells and tissue respond in the same way, a non-specific way, to all IAs, whether that be hypoxia, chemical agents, physical agents, infectious agents, immune responses, genetic abnormalities, or nutritional injury [47]. This concept is not only questionable, it is not consistent with the pathogenesis of a highly specific, complex disease. We believe atherosclerosis is too complex to be caused by multiple agents.

Chapter 2 showed marked differences in the histology of arteries injured by PTCA compared with recently placed coronary bypass vein grafts, or to atherosclerotic fibrous tissue (Figures A-E). The artery wall does not respond in a non-specific way to all IAs. Risk factors such as hyperlipidemia, smoking, and high blood pressure contribute to, accelerate, or aggravate the growth and expansion of the primary IA, but they are not the primary IA itself. These cardiovascular risk factors cannot be

the primary IA because all patients who have them do not develop atherosclerosis and many patients without them do develop atherosclerosis. We must look beyond cardiovascular risk factors for the primary IA.

Atherosclerosis may be caused by a single agent, a single mutation, a family of similar agents, or possibly through molecular mimicry [166,167]. For example, Benditt has shown the SMCs of atherosclerotic plaques to be monoclonal in origin and suggested that these monoclonal cells arose out of a single SMC mutation, possibly caused by chemical mutagens or viruses [12,168]. Monoclonality of SMC in atherosclerotic plaques serves to focus attention on the possibility that atherosclerosis may be caused by single rather than by multiple IAs.

Mechanism of Progression

An understanding of how the IA spreads, expands, and progresses is fundamental to characterizing it. What is the driving force behind the growth and expansion of atherosclerotic plaques and the accumulation of excessive numbers of SMCs [12]? What is the source of energy that sustains and/or replenishes the IA? Does the initiating IA continue to be present and active, increasing in number, amount, and concentration as the disease progresses? If so, by what sustaining mechanism? What is the mechanism of continuing injury? Is it caused by the same IA responsible for the initial injury? Is continuing injury due to the presence of cellular toxins, such as oxidized LDL, generated by a series of metabolic and biological reactions set in place by the original injury, which then become self-perpetuating [169]? If so, it is akin to a vicious cycle that once set in motion is difficult or impossible to stop or interrupt

[18]. Does oxidized LDL beget oxidized LDL, injuring more and more tissue, and causing growth and expansion of plaques?

Atherosclerosis is a progressive disease, but not necessarily steadily progressive. It is marked by exacerbations and remissions that can be greatly influenced by the control of risk factors, particularly the reduction of blood lipids [170–172]. A disease subject to exacerbations and remissions is not consistent with a disease driven in a relentless, self-perpetuating circle. If this reasoning is correct, the progression of atherosclerotic lesions is not due to a self-perpetuating chemical or metabolic reaction. It is due to a series of highly specific cellular and molecular responses, caused by an IA that may be considerably influenced by external factors.

Progressive growth and expansion of atherosclerotic injury can only be explained and can only occur if the causative IA is able to replicate and/or be continually replenished. Lee, et al, notes lipids may become “biologically active,” suggesting lipids have a life of their own, are capable of replication, or are self-replenishing [63]. If the IA is not continually replenished by some metabolic mechanism, such as occurs in a vicious circle, but expands by virtue of replication of the IA, then the IA may be an infectious organism. Furthermore, if the growth and progression of atherosclerotic lesions are decreased or reduced by reducing circulating lipid, and if the IA responsible for this growth is an infectious organism, then the infectious organism may require lipid for survival, growth, expansion, and replication. Pathogen-infected cells may alter membrane traffic for nutrient acquisition or act as a cofactor to lipids in atherosclerosis [173,174].

Evolutionary Purpose

What is the evolutionary purpose of lipid retention in atherosclerosis? In Figures 1–4, Chapters 1 and 2, we demonstrated the appearance of lipid-laden SMC in early atherosclerotic lesions, and also noted lipid-laden SMC are not present in normal, unaffected intima. Lipid-laden SMCs must have been altered or affected in some specific way, presumably by the IA, to cause them to take up excessive amounts of lipid. In vitro studies show infectious agents can alter biological processes in the artery wall and predispose to atherosclerosis [165]. The IA has either entered the SMC and altered intracellular mechanisms concerned with lipid regulation, or factors external to the cell, associated with the IA, have altered these mechanisms and produced a dysfunctional but still viable cell [175,176]. If the IA were an extracellular toxin, such as oxidized LDL, we would expect the cell to be destroyed, not to be rendered partially dysfunctional. The same holds true for cellular injury caused by other classes of IAs, including hypoxia, physical agents, and nutritional injury, which are unlikely to alter intracellular function in such a specific way without killing the cell. Furthermore, there is no reason for chemical or physical agents, hypoxia, or nutritional injury to promote the retention of lipid or the uptake of lipid by the SMC because the retention of lipid has no effect on the action of these agents. For these reasons, we do not believe the IA causing atherosclerosis is an extra-cellular toxin, chemical, or metabolite, but is an intracellular infectious organism that is able to alter intracellular mechanisms to suit its own intended purposes.

Herpes simplex virus (HSV), Cytomegalovirus (CMV), Chlamydia Pneumoniae (Cp), and Helicobacter Pylori (H. Pylori) are four organisms currently being considered in the pathogenesis of atherosclerosis. Studies show these four organisms can target and infect SMC, macrophages, and endothelial

cells, and can alter intracellular mechanisms involved in the uptake, metabolism, and degradation of cholesterol, with resulting lipid-laden cells [177–179]. The lipid-laden SMCs in Figures 1–4 may all be infected with an infectious organism that specifically alters intracellular metabolism concerned with the uptake of lipid, presumably for some purpose beneficial to the IA. For example, Cp is an energy parasite that utilizes host cell mechanisms to supply adenosine triphosphate [174]. The uptake of lipid by an SMC infected with Cp may be caused by or promoted by the organism to secure an energy supply from the ingested lipid [174,177]. Cp may stimulate the expression of scavenger receptors by the SMC to take up oxidized LDL, in the same way *H. Pylori* stimulates the formation of iron-scavenging systems [180]. Lipid retention in the ECM, and the excessive uptake of lipid by the SMCs may be orchestrated by Cp or other organisms for their own benefit [174].

The driving force behind the growth and expansion of the atherosclerotic lesion may be a replicating, expanding, growing, infectious organism. We speculate the infectious organism continues to be present, active, and replicating as long as an adequate supply of the necessary lipid is available to it. Restriction or lowering of blood lipid may affect lipid metabolism within the plaque as well as the availability of the type of lipid required by the organism. This could explain why lesion growth is retarded and acute events decreased in those patients whose serum lipids are reduced [170–172].

Based on the evidence presented, we hypothesize that atherosclerosis is caused by an infectious organism that alters intracellular functions and mechanisms, creating an increase in lipid uptake and retention by SMCs. The intracellular infectious organism then utilizes the retained lipid, either directly or indirectly, as a source of energy to fuel replication, growth, and expansion of atherosclerotic lesions. Eventually it infects other SMCs,

macrophages, and endothelial cells, causing the same intracellular abnormalities in each infected cell.

Single versus Multiple Infectious Agents

If the IA responsible for initiating atherosclerosis is an infectious organism, is it a single infectious agent, a single family of agents, or multiple, different, infectious agents that are able, through molecular mimicry, to produce similar types of injury (166,167)? The objections raised earlier in this chapter to multiple IAs causing the same series of complex cellular and molecular changes in the artery wall also apply to multiple and different infectious agents. It is unlikely that different types of infectious agents, such as Cp, HSV, CMV, *H. Pylori*, or other unknown infectious agents, could all produce exactly the same cellular changes and lead to the same atherosclerotic lesions in all patients. The possibility exists atherosclerosis may be caused not only by a single injurious agent, but a single infectious organism [181].

A single infectious organism entering the SMC and altering specific intracellular functions could be responsible for the highly specific cellular and molecular responses of plaque formation and the uniformity of atherosclerotic lesions [18]. A single infectious organism, circulating in the blood, entering the artery wall at any vulnerable or injured site, could explain the multicentric origin (Chapters 1 and 5) and the histologic similarity of widely separated lesions. The growth, contiguous expansion and spread of a single, replicating, infectious organism could explain the circumferential and longitudinal spread of the disease, the findings of infectious organisms within plaques [182], and the fusion of adjacent plaques. An infectious cause of atherosclerosis could explain the consistent presence of inflammatory cells surrounding plaques, and the

reason why atherosclerosis is a chronic inflammatory disease. A single infectious organism that infects and alters SMC function to such an extent that the cell ultimately dies could explain why plaque tissue degenerates and a necrotic core is formed. These observations provide insight into the nature of the IA, and lead us to advance the following hypothesis:

Hypothesis

We hypothesize that atherosclerosis is caused by a single infectious organism, endemic throughout the world [183–186]. Atherosclerosis is a world-wide disease, and its lesions are similar in all peoples, as with tuberculosis. We hypothesize that the infectious organism resides in a dormant state within a circulating cell, probably the monocyte exemplified by HSV, CMV, and Cp, as an obligate intracellular pathogen [187–189]. The organism is activated when the monocyte, acting as a vector, is attracted to sites of endothelial injury and carries the infectious organism into the artery intima [187,188,190].

We further hypothesize that the infectious organism, after entering the artery wall, is released from the monocyte enters and “infects” resident, intimal SMCs, and endothelial cells which now become an additional host cells [187,191]. The activated infectious organism is now present in monocyte derived macrophages, intimal SMCs, and endothelial cells [177], causing intracellular injury [183]. Considering the possibility that the various histologic features of atherosclerosis are infectious in origin raises questions about the mechanisms of injury.

Mechanisms of Injury

We postulate that the basic method or mechanism of injury utilized by the infectious organism is to enter and infect an intimal cell, injuring the cell and altering or subverting normal intracellular functions,

without killing the cell. Intracellular mechanisms are altered in ways that foster the replication, expansion, and spread of the infectious organism [183]. This particular mechanism of injury appears to be repeated over and over again, and is similar to the way a virus utilizes host cell mechanisms for replication [164,192]. The affected and infected cell then becomes a tool of the infectious organism, a component and participant in the disease process, rather than a defender against the infectious organism. These subverted intracellular mechanisms include, but are not limited to, the following:

1. Subversion of macrophage functions, resulting in failure of the macrophage to recognize, kill, and remove the infectious organism at the time of initial injury when the organism is present in small amounts and localized to a small area of the artery wall. The responsible intracellular mechanisms are not presently known, but defective killing of phagocytosed organisms is known to occur in chronic granulomatous disease [193]. This initial failure of macrophage function may explain how the infectious organism is able to establish a foothold, and “set up shop,” and create atherosclerotic lesions in multiple, separate areas of the artery wall. In conjunction with the failure to neutralize and kill the infectious organism, the macrophages also fail to phagocytose and remove dead and damaged tissue, as well as fail to participate in the repair and healing of the injured tissue [194]. Subversion of these 2 basic defensive responses, failure to phagocytose and kill the infectious organism, and failure to remove damaged tissue at the time of initial infection, is pivotal in the initiation of atherosclerosis.

2. Subversion of normal intracellular functions of the SMC results in the secretion of an abnormal form of CSPG that reacts with and retains lipid within the interstices of the extracellular matrix [10]. This subversion may explain why lipid is a major component

of atherosclerotic tissue [35]. The retention of lipid is a component, not a complication of atherosclerotic disease, and all plaque tissue containing such lipid is not normal tissue. It is diseased and pathologic. Early plaque growth is associated with proliferation of SMCs, not normal SMCs but SMCs altered in some way, inhibited from healing the area of injury [12].

3. The infectious organism subverts the intracellular mechanisms of the SMC, monocyte-derived macrophages, and endothelial cells governing the uptake, metabolism, and degradation of extracellular lipid, particularly the expression of scavenger receptors [167,175]. Disturbing, altering, or subverting these lipid regulatory mechanisms results in excessive uptake of lipid and results in the formation of lipid-laden SMC, macrophages, and endothelial cells. The purpose behind this lipid up take may be to allow the host cell, directly or indirectly, to metabolize the lipid into a form that can be utilized, such as oxidation of LDL and/or the esterification of cholesterol, by the infectious organism (195). Oxidized LDL may be one form of lipid required by the infectious organism [175].

4. The ingestion of excess lipid by intimal SMCs is associated with loss of cellular mobility within the tissue, limiting the ability to migrate and transport lipid back into the circulation [37]. Continued lipid ingestion, over and above that required by inherent cellular metabolism and/or the infectious organism, ultimately results in over-distention, rupture, and death of the cell. Death of the cell releases the retained lipid, and presumably the infectious organism itself, into the extracellular space. The infectious organism is now presumably free to infect other cells, but it is also exposed to the B and T lymphocytes of the immune system (192). The release of the infectious organism upon the death of the intimal cells may be the event that triggers activation of

the immune system and the proliferation of B and T lymphocytes, commonly associated with later, more advanced stages of atherosclerosis.

5. In some way, the infectious organism is able to subvert or to avoid the killing immune responses employed by T and B lymphocytes after the immune system is activated. As evidence in Chapter 3 showed, the number of T cells in the adventitia increased in direct proportion to plaque size, indicating active, continuing injury by the IA, along with generation of more and more antigen. Activation of the immune system leads to the proliferation of antigen-specific T lymphocytes, the number of T and B cells being directly related to the amount of the antigen produced or presented to the T cells [192,193].

However, activation of the immune system and the associated activation of T lymphocytes, although quite vigorous and progressive to judge by the number of T cells, is not effective in neutralizing the IA, or in halting the growth and spread of the disease. Plaques continue to grow and expand in spite of a vigorous immune response. If these immune responses were effective, growth and spread of the infectious organism would be stopped, plaque growth would cease, and the number of T cells would decrease. Although the T cell and other immune responses appear to be intact and functioning normally, the IA is able to avoid, subvert, or overcome these various defenses and survive, replicate, grow, and expand.

We gain some insight into this issue from the study of *H. Pylori*. *H. Pylori* is a well-adjusted parasite that survives in a hostile environment despite vigorous humoral and cellular immune responses against it [180]. The enzymatic pathways it needs for survival are continually switched on, and the presence of variable regions in gene coding for surface structure allows organisms to evade immune responses by

altering their surface antigens. This could be true in atherosclerosis if the infectious organism is able to subvert the immune system.

What immune mechanisms are subverted or altered to allow an infectious organism survive in atherosclerotic plaques? Insight is gained from studies of the Acquired Immunodeficiency Syndrome (AIDS) virus. The AIDS virus enters, subverts, and eventually destroys the helper T lymphocytes that play a key role in presenting viral antigens to the killer T lymphocytes [192,193,196]. Helper T lymphocytes secrete 2 cytokines, Interleukin-2 and Interferon-gamma, essential for the immune system to function normally and effectively [193,196]. If these two cytokines are absent, the immune response is blunted, killer mechanisms are compromised, infectious organisms are not killed, and the infectious organism continues to replicate, grow, and spread [196]. Something similar may happen in atherosclerosis, preventing the infectious organism from being neutralized and eliminated by the immune system.

6. An infectious organism subverts normal defensive responses by altering the hemostatic response to injury. Hemostatic abnormalities are produced by altering the phenotype of the endothelial cells from anticoagulant to procoagulant, generating the formation of thrombin [181]. SMCs and endothelial cells infected with Cp express PAI-1, T factors, and Interleukin-6, all procoagulants [197]. Excessively high levels of PAI-1 disturb the thrombo-regulatory balance between TPA and PAI-1, producing a prothrombotic state. The result is an increase in hemostatic responses to endothelial injury over and above that required for injury repair. If this action of the infectious organism does produce a prothrombotic state, to what purpose? The most logical explanation is that thrombus in some way facilitates

the growth and replication of the organism, possibly through increased or more readily available lipid [140].

Autoimmunity

Perhaps atherosclerosis is an autoimmune disease, and arterial wall injury is caused by immune complexes [166]. We visualize autoimmune disease as similar to a vicious circle. Once established, it is very difficult to stop, and it is not subject to the exacerbations and remissions seen with atherosclerotic disease. We would also visualize an autoimmune disease, caused by circulating immune complexes, to be a diffuse disease, not a multicentric, focal disease. Further, we would expect an autoimmune disease to respond to the administration of corticosteroids, but steroids have not been shown to affect the course of human atherosclerosis. It is possible that autoimmune mechanisms may play a role in contributing to the cellular injury and cell death within a plaque and in this way contribute to the expansion and spread of the IA.

In Review

Based on the evidence presented, we believe the primary IA causing atherosclerosis is a single infectious organism, an obligate intracellular pathogen residing in a circulating cell, probably the monocyte. Whether the organism is a bacterium or a virus is unknown. Both classes of agents appear to infect intimal cells and to alter intracellular mechanisms with the potential to produce atherosclerotic lesions. The infectious organism is activated after entry into the artery wall, infects intimal cells, and establishes a foothold within the wall. The organism proceeds to replicate, expand, and grow in all directions from a central focus. We speculate that the organism requires lipid, probably oxidized LDL, to fuel replication and growth, and that it subverts normal cellular functions and defense mechanisms to procure this

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lipid without killing the cell. We believe all of the abnormal cellular responses, listed above, are orchestrated by the infectious organism to procure the energy required for replication and growth.

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